Identification of *Tomato Mosaic Virus* Infection in Jasmine

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Abstract

Virus-like symptoms were recently observed on leaves of landscape and nursery downy and star jasmine (*Jasminum multiflorum*) and wax jasmine (*J. gracile*) in southeast Florida. Foliar symptoms included mottling, chlorotic ring spots and chlorotic line patterns. An agent was mechanically transmitted with difficulty from symptomatic leaves of downy jasmine to *Nicotiana debneyi* and *N. tabacum* 'Xanthi' and subsequently from these hosts to *Chenopodium quinoa* and other herbaceous test plants. Virions were isolated from *N. tabacum* 'Xanthi.' Rod-shaped particles (297 x 18 nm) similar to tobamoviruses were observed in partially purified virus preparations, and in leaf dips from symptomatic star jasmine and indicator plants. Extraction of viral-associated double-stranded (ds) RNA revealed a profile consistent with that of a tobamovirus. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis showed that the virus contained one polypeptide with an approximate molecular weight (Mr) of 18 kDa. The virus reacted specifically with IgG for *Tobacco mosaic virus* (TMV) and related tobamoviruses [including *Tomato mosaic virus* (ToMV)] in double antibody sandwich, enzyme-linked immunosorbent assay. No reaction was observed with TMV specific IgG. Reverse transcription-polymerase chain reaction with total RNA isolated from symptomatic jasmine leaves and infected *N. tabacum* 'Xanthi' using ToMV coat protein specific primers amplified the expected product from downy and star (but not wax) jasmine and *N. tabacum* 'Xanthi.' The nucleotide and amino acid sequence of the products were 100% identical to the corresponding fragment of a Brazilian isolate of ToMV from *Impatiens*. To our knowledge, this the first report of ToMV in jasmine in the USA.

INTRODUCTION

Among the different *Jasminum* species (*Oleraceae*), downy and star jasmine (*Jasminum multiflorum*) and wax jasmine (*J. gracile*) are commercially cultivated and commonly grown ornamentals in Florida. They are popular decorative shrubs widely planted around private gardens and public buildings for their long life, vigor and delicate smell from their flowers.

To date, three well-characterized viruses have been identified in different jasmine species, including 1) *Tobacco ringspot virus* (TRSV) in *J. nudiflorum, J. mesney* and *J. officinale* (Waterworth. 1971, 1975; Morton, et al., 1976), 2) *Arabis mosaic virus* (ArMV) in *J. mesney* and *J. officinale* (Waterworth, 1971; Cooper and Sweet, 1976), and 3) *Cucumber mosaic virus* (CMV) in *J. polyanthum* (Annual Report of Denmark, 1982). A virus named Jasminium chlorotic ring spot virus (JCRSV) found in *J. sambac* in India has been reported to be whitefly (*Bemisia tabaci*) transmitted (Wilson, 1972). In addition, two

more viruses, one called Jasmine mild mosaic from *J. multiflorum* showing mild mosaic symptoms and the other called Jasmine latent virus 1 from symptomless *J. odoratissium,* have been isolated on host plants (Waterworth, 1971) but not further characterized.

Virus-like symptoms were recently observed on the leaves of landscape and nursery downy, star and wax jasmine plants in southeast Florida. Foliar symptoms included mottling, ring spots and chlorotic line patterns (Fig. 1). The objective of this study was to identify the pathogen(s) associated with the disease symptoms observed on downy, star and wax jasmine plants.

MATERIALS AND METHODS

Plant Material, Inoculation Methods and Host Range Determination

Nursery propagated downy, star and wax jasmine plants with foliar symptoms suggestive of virus infection were maintained in a greenhouse. Tissue (leaf, bark and flower) samples from these plants and landscape grown plants were used for mechanical inoculations of herbaceous hosts. Jasmine tissues were triturated with various buffers containing chemicals such as ascorbic acid, caffeine, ethylendiaminetetraacetate (EDTA), nicotine sulfate, potassium cyanide, sodium diethyldithiocarbamic acid, sodium salt (DIECA), sodium sulfite, sodium thioglycolate, 2-mercaptoethanol (2-ME) and gelatin. Such tissue extracts were rubbed onto Carborundum-dusted leaves of herbaceous test plants from *Chenopodiaceae* and *Solanaceae* and inoculated leaves were rinsed with tap water. Inoculated plants were held in an air-conditioned greenhouse under natural lighting with a daytime temperature of 24° C and observed for symptom development. Following one of these inoculations, symptoms were observed on *Nicotiana debneyi* and *N. tabacum* 'Xanthi' plants. Subsequent mechanical transfer from these *Nicotiana* plants to a range of herbaceous test plants used leaf tissue triturated in 20 mM sodium phosphate buffer (pH 7.0) containing 1% (wt/vol) Celite. The isolated virus was maintained by serial passages on *N. tabacum* 'Xanthi' or *Chenopodium quinoa* Willd. plants.

Isolation and Characterization of Double-stranded (ds) RNA and Virions

Mechanically transmitted virus was propagated in *N. tabacum* 'Xanthi' and systemically infected leaves were harvested 14 days after inoculation. DsRNA was extracted from 7-g leaf samples following the protocol of Valverde et al. (1990). DsRNA was analyzed by electrophoresis on native 5% polyacrylamide gels and detected by silver staining.

Based on dsRNA analyses, a tobamovirus was suspected and a protocol described by Wetter and Conti (1988) was used for virion isolation. Virus concentration was estimated spectrophotometrically using an extinction coefficient of $E_{260nm}^{0.1\%} = 3.00$ Virions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12.5% SDS-PAGE gels and stained with Coomassie brilliant blue G-250 according to established protocols (Sambrook and Russell, 2001). For serological analysis, proteins in replicate gels were transferred following electrophoresis to polyvinylidene difluoride membranes as described by Ullman et al. (1993). Commercially available IgG (Agdia Inc., Elkhart, IN, USA) for *Tobacco mosaic virus* (TMV) and related tobamoviruses [including *Tomato mosaic virus* (ToMV)] was used at a dilution of 1:200 (vol/vol) for detection of viral antigen. Goat anti-rabbit alkaline phosphatase conjugate (Bio-Rad) at a concentration of 1:3000 was used as the secondary antibody. Nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrates were used as recommended by the supplier (Bio-Rad).

Double Antibody Sandwich, Enzyme-linked Immunosorbent (DAS-ELISA)

DAS-ELISA tests of partially purified virus preparations, infected herbaceous plants and jasmine tissues (leaves, bark and flowers) were performed as described by Clark and Adams (1977). Commercial antibodies and conjugates specific for TMV and related tobamoviruses (Agdia Inc.) were used at 1:200 (vol/vol) dilutions as

recommended by the manufacture. Controls in all ELISA assays included sample buffer only, and virus-infected and uninfected *C. quinoa* leaf tissue. Absorbance at 405 nm was measured with an MRX Revelation microplate reader (Thermo Labsystems, Chantilly, VA) about 90–120 min after the addition of the substrate (*p*-nitrophenyl phosphate). Samples with $A_{405\ nm}$ readings > 0.1 were considered positive, a reading which was always a minimum of four to five times the mean absorbance of uninfected (herbaceous plant) leaves.

Reverse Transcription-polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from 100 mg symptomatic, fresh jasmine leaf tissue using a QIAGEN RNeasy plant mini-kit (Qiagen, Crawley, UK), according to the manufacturer's instructions. A ToMV viral sense primer (5'-GTTCAGGCGGAAGGTCTAAACC-3') and a viral antisense primer (5'-AAATTTATATTTCAGCACCTATGCAT-3') were selected by computer analyses of the published nucleotide sequences of the coat protein region. 5 µl of total RNA was combined with 5 µl of DEPC-treated H_2O and 1 µl of ToMV-specific antisense primer and incubated at room temperature for 10 min. First strand cDNA was synthesized by MMLV reverse transcriptase (Promega, Madison, WI) at 45°C for 45 min with standard methods (Sambrook and Russell, 2001). Thirty cycles of PCR amplification with *Taq* polymerase (5 U/µl; Promega Corp.) at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min and 30 s in the manufacturer's buffer followed first-strand synthesis in the same tube. The predicted length of the amplified DNA product was 480 bp. Products were analyzed by electrophoresis on native agarose gels and detected by ethidium bromide staining. Products of the expected size were excised and purified using a QIAquick PCR purification kit (Qiagen, Crawley, UK) and ligated into pGEM-T (Promega). Selected clones were sequenced on an ABI3700 automated sequencer at the USHRL Genomic Laboratory. Sequences were edited in VectorNTI (InforMax, North Bethesda, MD) and subjected to BLAST searches (Altschul et al., 1997).

RESULTS AND DISCUSSION

In repeated mechanical inoculations, a virus was transmitted from downy jasmine leaves on two occasions (once to *N. debneyi* and once to *N. tabacum* 'Xanthi') by the use of 0.01 M borate buffer (pH 7.8), containing 0.01 M EDTA, 0.01 M sodium sulfite (Hicks and Frost, 1984) with addition of 0.1% 2-ME. In both cases, 12-14 days after inoculation a single chlorotic spot developed on one of the inoculated leaves. Inoculations of *C. quinoa* plants with sap from symptomatic leaves of *N. debneyi* and *N. tabacum* 'Xanthi' resulted in the development of chlorotic lesions with necrotic centers, followed by systemic infection several days later. Because identical symptoms were observed on *C. quinoa* inoculated with the two isolated viruses, subsequent mechanical inoculations were made with only one of them. Electron microscope examination of sap from symptomatic *C. quinoa* plants revealed particles resembling those of tobamoviruses. Based on that finding, the experimental host range was determined by mechanical inoculations of plants known as hosts of solanaceous-, brassica-, cucurbit- and malvaceous-infecting subgroups of tobamoviruses using sap from infected *C. quinoa* and later, partially purified virus preparations. No infection was obtained with any of the inoculated plants from *Brassicaceae, Fabaceae, Malvaceae* and *Cucurbitaceae*, excluding *Cucumis sativus* L. "Straight Eight" which reacted with chlorotic lesions on inoculated leaves. The inoculated plants from *Solanaceae,* as well as *Gomphrena globosa L.* (*Amaranthaceae*), however, showed symptoms characteristic for ToMV infection. Systemic mosaic on *Gomphrena globosa* L. and only local necrotic lesions on *Capsicum annuum* L., *Datura stramonium* L., *N. glutinosa* L., *N. sylvestris* Spegaz. & Comes and *N. tabacum* 'Xanthi nc' were recorded. The symptoms induced on *Lycopersicon esculentum* Mill. (dark-green systemic mosaic), *N. benthamiana* Domin. (local necrotic lesions; systemic vein clearing, stem necrosis and plant death), *N. clevelandii* A. Gray (local necrotic lesions; systemic mosaic, leaf deformation), *N. debneyi* (local necrotic lesions; systemic mosaic), *N. tabacum*

'Xanthi' (local chlorotic lesions; systemic vein clearing, mosaic and necrotic etching), *Petunia* × *hybrida* (local necrotic lesions; systemic vein and stem necrosis and mosaic) were identical to the symptoms observed on these plants when inoculated in parallel with a tomato isolate of ToMV from Florida. Similar symptoms on the above solanaceous host plants have been described by Jacobi et al. (1992) for a red spruce isolate of ToMV. In contrast, systemic mottling and necrotic lesions on *N. rustica* L. induced by the jasmine ToMV isolate differed from the symptoms (only local lesion) on that plant induced by ToMV-L, ToMV-Dahlemense and ToMV-DW isolates (Reddick, 1989). Unlike, the ToMV-DW isolate, the ToMV jasmine isolate, as well as ToMV-L and ToMV-Dahlemense isolates (Reddick, 1989) induced systemic mosaic symptoms on *Lycoipersicon esculentum* L.

A dsRNA isolated from systemically infected leaves of *N. tabacum* 'Xanthi' co-migrated with a dsRNA isolated from the same host infected with a tomato isolate of ToMV and a TMV dsRNA marker (Fig. 2).

An average virus yield of 40mg/100g of fresh tissue (*N. tabacum* 'Xanthi') was obtained. Electron microscope analysis of the resulting virus preparations indicated the presence of rod-shaped particles with a mean length of 297 nm (Fig. 3B), indistinguishable from those observed in the leaf dips from infected *N. tabacum* 'Xanthi' tissues and symptomatic star jasmine leaves (Fig. 3A) and typical of those described for tobamoviruses. Average particle lengths of 296 nm for ToMV-DW isolate (Reddick, 1989) and 270-300 nm for ToMV isolated from water draining forest stands (Jacobi and Castelo, 1991) have been reported.

A single Coomassie blue-stained band was observed after SDS-PAGE analysis of purified virus preparations (Fig. 4A). The coat protein had a molecular weight of approximately 18 kDa and co-migrated with that of a tomato ToMV isolate used as a control. Both the jasmine and tomato ToMV proteins reacted with IgG for TMV and other tobamoviruses in Western blot analysis (Fig. 4B).

In DAS-ELISA tests, tissue samples from downy and star jasmine (leaves, flowers and bark), infected *N. tabacum* 'Xanthi' and purified virus preparations reacted positively. $A_{405\ nm}$ readings of infected downy and star jasmine tissues ranged from 0.177 to 0.309 and those of infected leaves of *N. tabacum* 'Xanthi' and purified virus preparations were greater than 3.5. The results obtained in DAS-ELISA indicated that ToMV was present in almost equal concentrations in all tested jasmine tissues. No ToMV was detected by DAS-ELISA in any of the tested tissues (leaves, flowers, bark) of wax jasmine.

Fifteen jasmine samples were collected from different locations in southeast Florida (Fort Pierce, Port Saint Lucie, Lake Alfred, Mims, Fort Lauderdale, Miami) and tested for ToMV by DAS-ELISA. Thirteen samples from downy and star jasmine were positive for ToMV, whereas two samples from wax jasmine were negative.

Representative clones of RT-PCR products from total RNA of symptomatic downy and star jasmine were sequenced. The nucleotide and amino acid sequences of two clones (one from downy and one from star jasmine) were 100% identical to the corresponding coat protein fragment of a Brazilian isolate of ToMV from *Impatiens* (GenBank Accession No. AY063743 and No AAL38654.1).

CONCLUSIONS

The virus isolated from downy jasmine was identified as ToMV based on host range, symptoms, and serological and molecular analysis with supporting evidence from electron microscopy. ToMV infection was also identified in star (but not wax) jasmine by serological and molecular analysis.

ToMV is associated with diseases in a wide variety of annual and perennial plants. With the detection of ToMV on downy and star jasmines, its wide host range has increased. To our knowledge, this is the first isolation and first identification of ToMV in jasmine in the USA.

At this stage of our investigations, it is not clear whether the symptoms observed on downy and star jasmine were caused only by ToMV. Presently, the lack of uninfected jasmine plants prevented us from fulfilling Koch's postulates and reproducing the disease symptoms on jasmine plants by inoculation with the purified virus.

All three jasmines reacted strongly with *Tomato ringspot virus* IgG, apparently due to cross-reaction with plant protein(s) as we were unable to confirm this result with other means.

Since similar foliar symptoms were observed also in wax jasmine, in which ToMV was not identified by the methods used, the potential for infection by an additional virus should be considered. Further investigations are in a progress.

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Figures

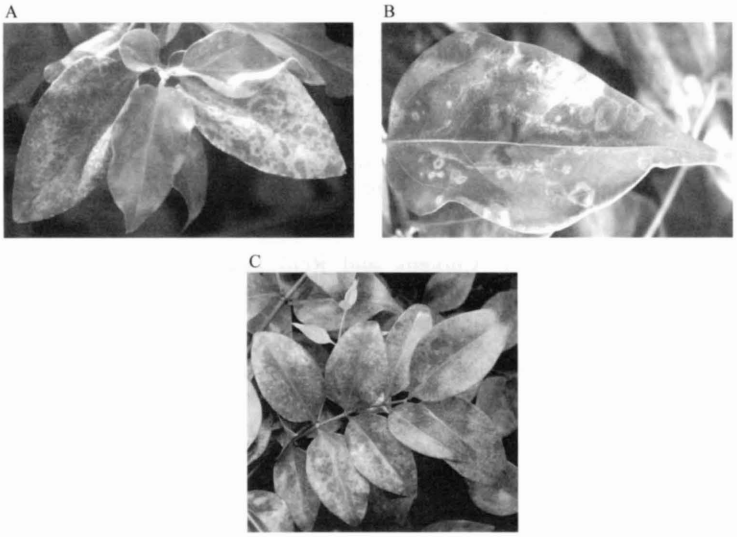

Fig. 1. Virus-like symptoms consisting of mottling, chlorotic ring spots and line patterns on leaves of (A) downy, (B) star and (C) wax jasmine.

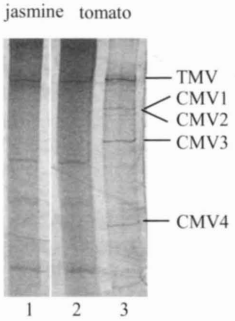

Fig. 2. *Tomato mosaic virus* (ToMV) characterization by viral-associated double-stranded (ds) RNA analysis. Lanes 1 and 2 contain dsRNA of jasmine and tomato isolates of ToMV, respectively. Lane 3 contains a mixture of *Tobacco mosaic virus* 5TMV) and *Cucumber mosaic virus* (CMV) dsRNAs.

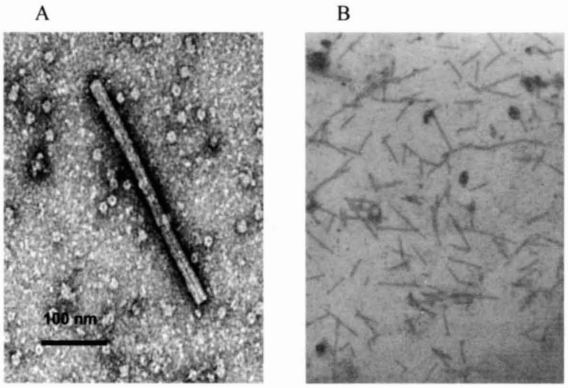

Fig. 3. Electron micrographs showing *Tomato mosaic virus* particles in (A) dip preparations from star jasmine, (B) purified from *N. tabacum* 'Xanthi.' Mean particle length of two hundred measured virions was 297 nm. Scale bar represents 100 nm.

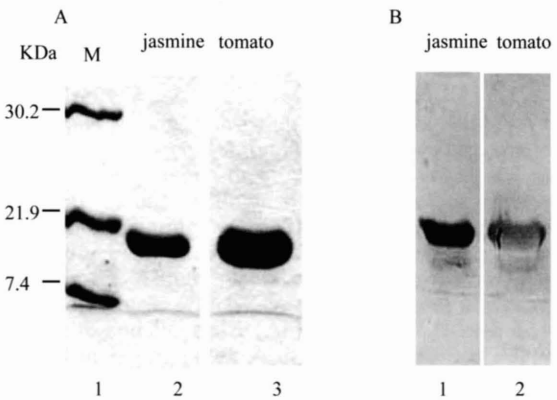

Fig. 4. (A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of jasmine *Tomato mosaic virus* (ToMV) isolate (lane 2). The coat protein co-migrated with that of tomato ToMV isolate (lane 3). The positions of pre-stained molecular weight markers (M) (lane 1) are indicated to the left of the gel. (B) Western blot of a replicate gel to that shown in (A) was probed with IgG against *Tobacco mosaic virus* and related tobamoviruses, including ToMV (Agdia Inc., Elkhart, IN, USA).